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**Preliminary investigation of genetic capture-mark-recapture to census bowhead whales (*Balaena mysticetus*) in Nunavut, Canada**

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## Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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## TABLE OF CONTENTS

ABSTRACT.....	iv
RÉSUMÉ .....	v
INTRODUCTION .....	1
METHODS.....	2
Sampling.....	2
Genetic Analysis .....	2
Error Rates.....	4
Recaptures.....	4
Within-location within-year estimates .....	4
RESULTS .....	4
DISCUSSION.....	6
ACKNOWLEDGEMENTS .....	7
LITERATURE CITED.....	8

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## ABSTRACT

Cost effective and efficient methods to census marine mammals are needed to monitor and manage populations. In bowhead whales, aerial surveys are commonly used but can be cost prohibitive and may not be capable of tracking individual animals unless coupled with photographic identification. Genetic capture-mark-recapture (gCMR) techniques are being used more often and may be able to both estimate local population size and to track animals as they move throughout the population's range. This research summarizes recent gCMR efforts on bowhead whales in the eastern Canadian Arctic and presents preliminary local abundance estimates. 714 skin samples have been collected sporadically over multiple years and locations. Collections were obtained during spring and summer from either boats or the floe edge and crossbow deployed 40- or 60mm biopsy darts. DNA was extracted and used to determine sex, mitochondrial haplotype, and to generate a microsatellite loci profile for all samples. Removing animals that were sampled multiple times resulted in samples from 642 individual whales sampled between 1995 and 2012. Although the long-term objective is to use the data to generate a population estimate, the current report summarizes recaptures among years and location and preliminary abundance estimates are limited to Foxe Basin (593 and 230 in 2009 and 2012, respectively) and Cumberland Sound (188 and 566 in 2011 and 2012, respectively). The simple local-annual estimates reported here are not useful for management purposes but serve to illustrate the utility of the proposed approach. Future research plans include conducting a more sophisticated capture-mark-recapture abundance analyses on these data to generate demographic models. Adding more recent samples, such as from 2013, and including data from west Greenland could lead to a robust abundance estimate for the eastern Canadian-west Greenland bowhead whale population.

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**Enquête préliminaire sur les études de capture-marquage-recapture génétique pour le recensement des baleines boréales (*Balaena mysticetus*) au Nunavut, au Canada**

**RÉSUMÉ**

Il nous faut des méthodes économiques et efficaces pour recenser les mammifères marins afin de surveiller et de gérer les populations. Pour la baleine boréale, on emploie souvent des relevés aériens, mais ceux-ci peuvent être trop coûteux et ne permettent pas toujours de suivre un animal donné, sauf s'il est complété avec une identification photographique. On utilise de plus en plus souvent des techniques de capture-marquage-recapture génétique (CMCg), celles-ci permettant à la fois d'estimer la taille des populations locales et de suivre le déplacement des animaux dans l'aire de répartition de la population. Cette étude résume les activités récentes de CMCg menées sur la baleine boréale dans l'Arctique canadien de l'Est et présente une estimation préliminaire de l'abondance locale. On a prélevé 714 échantillons de peau sporadiquement sur plusieurs années et à divers endroits. Les prélèvements ont été effectués au printemps et en été à partir de bateaux ou bien au bord de la banquise, à l'aide de fléchettes de biopsie de 40 ou 60 mm tirées à l'arbalète. On a ensuite extrait l'ADN afin de déterminer le sexe de l'animal, son haplotype mitochondrial, et pour générer un profil de locus microsatellite pour chaque échantillon. En éliminant les animaux dont on avait prélevé de multiples échantillons, on a calculé que l'on disposait d'échantillons de 642 baleines différentes obtenus entre 1995 et 2012. Même si l'objectif à long terme est d'utiliser ces données pour calculer une estimation de la population, le rapport actuel résume les recaptures de toutes les années et de tous les endroits, et les estimations d'abondance préliminaires se limitent au bassin Foxe (593 et 230 en 2009 et 2012, respectivement) et à la baie Cumberland (188 et 566 en 2011 et 2012, respectivement). Les simples estimations locales annuelles indiquées ici ne sont pas utiles pour la gestion des populations, mais elles illustrent l'intérêt de l'approche proposée. Les plans de recherche future comprennent des analyses de l'abondance plus complexes d'après ces données de capture-marquage-recapture afin de créer des modèles démographiques. L'ajout de nouveaux échantillons plus récents, comme ceux de 2013, et l'inclusion de données du Groenland occidental pourraient permettre d'obtenir une estimation rigoureuse de l'abondance de la population de baleines boréales de l'est canadien et de l'ouest du Groenland.

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## INTRODUCTION

Bowhead whales (*Balaena mysticetus*) are baleen whales which are listed by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) with a status of special concern (COSEWIC 2009). Bowhead whales were heavily hunted between the 16<sup>th</sup> and 19<sup>th</sup> century with an estimated 77 000 animals taken (Higdon 2010). This whaling pressure reduced the population to historic low levels in the 1800s (Mitchell and Reeves 1981). From this low point, populations have recovered to an uncertain level and are now subject to limited hunting (Heide-Jørgensen et al. 2010) by Canadian Inuit and Greenlanders. In Canada, the sustainable co-management of this resource is dependent on accurate population estimates, which are challenging and expensive. Aerial surveys have traditionally been used to estimate population size of bowheads but a number of factors (i.e., whale rarity, large area to survey, and undetected diving whales while survey planes are flying overhead) have increased the need for additional estimating methods (Koski and Ferguson 2012).

Four bowhead whale populations are delimited for the circumpolar Arctic and within Canadian waters, two populations occur: Bering-Chukchi-Beaufort and Eastern Canada-West Greenland (EC-WG). The EC-WG ranges throughout the waters of the eastern Canadian Arctic, Baffin Bay, Hudson Strait, and Foxe Basin. Within this range there is evidence of age, sex, and reproductive class segregation (Heide-Jørgensen et al. 2010). For example, female bowhead whales (cows) and their calves make up a large portion of the whales observed in northern Foxe Basin each spring (Cosens and Blouw 2003). It has been suggested that this area acts as a refuge from killer whales and reduces predation on calves as well as providing access through Fury and Hecla Strait to the central Arctic (Hay et al. 2000, Cosens and Blouw 2003).

Appropriate harvest levels are set based on population estimates but the surveys to determine abundance can be logistically difficult and expensive to conduct. Alternate, or complimentary, methods to estimate population size are needed. Several options to estimate abundance are available: aerial surveys of summer or overwintering areas, mark-recapture estimates from photographic surveys, mark-recapture estimates from genetic sampling, vessel- or shore-based surveys, and acoustic surveys (Koski and Ferguson 2012).

Capture-mark-recapture (CMR) techniques use marking or identification of individuals to estimate abundance and other population parameters, such as birth, death, immigration, and emigration rates (DeMaster et al. 1980, White and Burnham 1999). In a fashion similar to traditional capture-mark-recapture techniques, genetic CMR (gCMR) use the unique genetic fingerprint of each animal as an identifying mark (Palsbøll et al. 1997, Petit and Valiere 2006). The use of genetic identifications to 'mark' animals have been used on a variety of taxa (e.g., bears (Mowat and Strobeck 2000) and NA right whales (Frasier et al. 2009)) using a variety of samples sources (hair (Mowat and Strobeck 2000), scat (Kohn et al. 1999), and skin biopsies (Wiig et al. 2011)). General reviews of this methodology can be found in the literature (e.g., White and Burnham 1999, Lukacs and Burnham 2005). Genetic profiles have been successfully used to estimate population size in cetaceans (e.g., Stevick et al. 2001, Garrigue et al. 2004, Baker et al. 2012) including in bowhead whales (Wiig et al. 2010, Wiig et al. 2011). Wiig et al. (2011) calculated an estimated of 1410 (95% CI 783-2038) bowhead whales for the Disko Bay region based on their mark-recapture analysis however they also identified a high degree of sex and reproductive class segregation and thus this estimate is likely only a portion of the population (Koski and Ferguson 2012).

Genetic CMR (gCMR) has the potential to provide a unbiased population estimate because every animal that is captured is marked (no unmarked animals) and that animals do not lose marks over time (Stevick et al. 2001). However, all CMR estimates including gCMR as subject



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to biases if the capture probability varies across groups (e.g., by sex). This is known to be the case for bowhead whales (see Finley 1990, Cosens and Blouw 2003, Heide-Jørgensen et al. 2010). Additionally, without visual identifications there may be some oversampling (i.e., same animal is sampled multiple times within a single sampling period) and there is some quantifiable error in the generation of genetic data (McKelvey and Schwartz 2004). Koski and Ferguson (2012) convened a workshop to evaluate methods of abundance estimation for the eastern Canadian Arctic and determined that gCMR was potentially the lowest cost approach the extended time span for sample collection and the difficult sampling in some areas, were drawbacks.

This research had several objectives: firstly, between-years within-location and between-years between-locations recaptures were examined to determine if sampling throughout the Arctic would result in sufficient recaptures to calculate a population estimate for specific locations and/or the entire EC-WG population (respectively). Although both types of recaptures carry equal weight in the model, between-years within-location recaptures along with field data may provide additional information about the biology of this species (e.g., birth interval). Secondly, within-year recaptures were examined to evaluate oversampling and local abundance. The latter assumes that the sampling area is closed during the sampling period and between-day recaptures can be used to estimate local abundance. The evaluation of this assumption that these areas represent closed seasonal populations can be evaluated in the future using preliminary satellite telemetry data. Thirdly, the issue of within-day oversampling is addressed.

## **METHODS**

### **SAMPLING**

Field teams searched for whales from either boats or from along the floe edge near communities. During most years one or two teams searched for bowhead whales based on local ice conditions and advice from local guides. In some years the field notes included a variety of additional data that could include approximate size, age or reproductive class, group size or composition, and other relevant notes. At the same time photos for photo ID were taken, satellite tags were deployed, and a skin biopsy was obtained. Biopsy samples were obtained using a 40- or 60mm biopsy dart (CETA-DART, Denmark) deployed with a 150lb crossbow (Excalibur Vixen, Ontario, Canada). Bolts (CETA-DART, Denmark) were equipped with a fluorescent coloured float to facilitate retrieval. In the field, samples were wrapped in aluminum foil then placed into liquid nitrogen until being transferred to -80°C freezer at the Freshwater Institute (DFO, CA, Winnipeg) laboratories until further analysis.

Intensive biopsy sampling programs have been conducted from Igloolik and Pangnirtung, Nunavut. In addition, samples used here include smaller collections from Repulse Bay and Arctic Bay and some samples were collected from harvested whales from annual subsistence hunts (e.g., Kugaaruk) (Table 1). Sampling was conducted with community members as part of the sampling team.

### **GENETIC ANALYSIS**

DNA was extracted using a modified version of the standard Qiagen extraction protocol (Frasier et al. 2009). This included extra steps to digest the tissue fully before extraction using Qiagen BioSprint 96 DNA Blood Kits (Qiagen Inc. Canada, Toronto, Ontario). DNA was quantified using a  $\mu$ Quant spectrophotometer (Bio-Tek US, Winooski, VT) and normalized to 10-100ng/ $\mu$ l. DNA was then used to amplify several genetic markers briefly outlined below.

Sex was determined through amplification of zinc finger protein intron using LGL331 and LGL335 primers (Shaw et al. 2003). PCR product was stained with GelRed (Biotium Inc., Hayward, CA) and visualized on a 1.5% agarose gel. The banding pattern of X (~975 bp) and Y (~1040 bp) fragments were used to infer sex.

*Table 1. Total number of bowhead whale samples collected at eastern Canadian Arctic locations and used in this study. Bold samples are used in the detailed analyses – see text for rationale.*

Year	Arctic Bay	Igloolik	Kugaaruk	Pangnirtung	Repulse Bay	Total
1995		10				10
1996		17				17
1997		1		17	5	23
1998					3	3
2000					4	4
2001		34	2		3	39
2002		53	5	10		68
2003		27				27
2004				6		6
2005				15	1	16
2006				31		31
2007		45				45
2008	2	6		3	5	16
2009	6	82			2	90
2011		41		53		94
2012		115		110		225
Total	8	431	7	245	23	714

Twenty-four microsatellite loci were optimized into multiplexes and run on an ABI 3130xl Genetic Analyzer (Life Technologies Inc., Carlsbad, CA). Microsatellites were multiplexed as follows: multiplex 1 with Bmy1 (1.6µM), Bmy8 (0.2µM), Bmy16 (0.6µM), EV37 (0.16µM), and EV104 (0.4µM); multiplex 2 with Bmy10 (0.4µM), Bmy55 (0.6µM), EV76 (0.2µM), FCB4 (0.3µM), and RW31 (1.2µM); multiplex 3 with Bmy19 (0.3µM), Bmy33 (0.4µM), Bmy36 (0.6µM), Bmy53 (1.6µM), and Bmy54 (0.4µM); multiplex 4 with Bmy49 (0.8µM), Bmy58 (1.0µM), and RW18 (0.3µM); and multiplex 5 with Bmy11 (0.3µM), Bmy57 (0.12µM), and EV1 (1.6µM). Three microsatellites were incompatible for multiplexing and were run individually. These were Bmy2, Bmy26, and GATA098 (0.5µM each).

In addition to primers the PCR reactions included 10X AmpliTaq Gold Buffer (1X), MgCl<sub>2</sub> (2.0mM), dNTP mix (0.25mM), AmpliTaq Gold DNA Polymerase (0.5U)(Life Technologies Inc., Carlsbad, CA) and nuclease-free water to a final volume of 10µl, including 1.0µl of template DNA.

Thermal cycler profiles included a 5 minute incubation at 95°C followed by 35 cycles of 95°C for 30 seconds, annealing temperature for 30 seconds, and 72°C for 30 seconds. The final extension was at 72°C for 30 minutes. Multiplexes 1, 2, 3, and Bmy26 had an annealing temperature of 55°C; multiplexes 4, 5, and Bmy12 had an annealing temperature of 58°C; and GATA098 at 48°C. Samples were run on an Applied Biosystems 3130xl Genetic Analyzer (Life Technologies Inc., Carlsbad, CA) with GeneScan 600 LIZ size standard (Life Technologies Inc., Carlsbad, CA) and Hi-Di formamide (Life Technologies Inc., Carlsbad, CA). The program GeneMarker (SoftGenetics, State College, PA) was used to analyze and score the raw microsatellite data.



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## ERROR RATES

Error rate was determined for the 2008 to present samples through the use of replicate samples. Five positive control samples were profiled between three and eight times in independent reactions of the complete microsatellite set. A further 34 samples were profiled twice in independent reactions. The error rate was calculated as the number of allele changes per number of alleles typed for each locus.

As the microsatellite data set spans several changes in technology and technicians, the older microsatellite data needed to be adjusted to be comparable to recently profiled samples. To do this, 46 random samples from before 2008 (2008 to 2012 are the most recent data set) were selected and these samples were re-extracted and re-profiled. These samples were then compared to determine a correction factor for each locus. The correction factor was then applied to the entire pre-2008 data set. This process also highlighted samples where there were errors and this correction factor error was calculated by identifying the number of alleles per locus where the correction factor deviated from the median correction factor.

## RECAPTURES

The program GeneCap vers 1.4 (Wilberg and Dreher 2004) was used to identify matching samples that indicated a recapture event. This program matches samples at each loci and for those that do match, it calculates the probability that they are similar to chance alone based on the allele frequencies of the entire data set. This probability was calculated using the more conservative method that assumes that siblings may be present in the dataset (Waits et al. 2001, Wilberg and Dreher 2004) and a match probability of 0.05 was used to create capture histories. This capture history can then be used in the program MARK (White and Burnham 1999) to calculate population estimates.

## WITHIN-LOCATION WITHIN-YEAR ESTIMATES

Estimates the number of whales in the waters of Foxe Basin near Igloolik and in Cumberland Sound was calculated for each of these locations for each sampling year using a simple Chapman estimator (Chapman 1951). This estimator is a modified Lincoln-Petersen estimator that adjusts for small sample size. Population size is calculated according to the following formula,  $N = [((M + 1)(C + 1))/(R + 1)] - 1$  where  $M$  = animals marked in the first sampling period,  $C$  = animals captured in the second sampling period, and  $R$  = animals marked in the first period and recaptured in the second period. We divided each field season with a between-day recapture into two periods based on the day of the first between-day recapture. Period one included all animals 'marked' up to the day before the first recapture. No estimate could be obtained for years when there was no recaptures. We recognize that this simple treatment of the data can be significantly improved with more sophisticated methods (e.g., multi-state mark-recapture (Calvert et al. 2009)). However, our intention is to provide preliminary results that can be used to evaluate efficacy of the method.

## RESULTS

714 samples were collected from Arctic Bay, Kugaaruk, Igloolik, Pangnirtung, and Repulse Bay were used in this analysis. Samples were collected between 1995 and 2012 although effort fluctuated among years and locations (Table 1). Detailed analysis is reported for northern Foxe Basin (Igloolik: 2007, 2008, 2009, 2011, 2012) and Cumberland Sound (Pangnirtung: 2011, 2012) as these years represent the years with increased effort to obtain biopsy samples coupled with an updated and expanded genetic profiling regime.

Sex and mitochondrial haplotype were used to confirm matched samples and for samples where these additional identifiers were available there were no mismatches. The final microsatellite data set contained 21 loci because three loci were dropped due to a significant portion of the samples missing data in the samples profiled before 2008. A sample was only included analysis if it had at least 10 of the 21 loci scored. This provided an overall probability of identity of  $1.11 \times 10^{-9}$  which corresponds to a 1 in 901 409 147 (or  $9.01 \times 10^8$ ) chance that the match identified was due to two random animal matching at all loci. This threshold could be reduced to include more samples. For example, Wiig et al. (2011) used eight loci in total with a threshold of four loci.

GeneCap identified 642 individuals in this dataset. Matched samples correspond to 41 within-location within-year recaptures, 13 within-location but between-year recaptures, and six between-year and between-location recaptures<sup>1</sup>. The length of time between recaptures at a location ranged from one to eight years (mean 4 years) and the length of time between recaptures at different locations was three to ten years (mean 7.8 years). Between-location recaptures were all the result of an animal that was marked in one location being recaptured in Cumberland Sound in a subsequent year. This pattern was observed between Igloodik and Pangnirtung (n = 3; 2 male, 1 female), Kugaaruk and Pangnirtung (n = 1; female), and Repulse Bay and Pangnirtung (n = 2; both female).

Detailed analysis of samples collected in Foxe Basin near Igloodik (2007-2012; n = 289; 264 individuals) revealed 30 matches, of which 12 matches were on the same day of the same year (potentially oversampling), eight were within the same year but on separate days, and four were between years. In 2009 there was a single within-year, between-day recapture and in 2012 there were six within-year, between-day recaptures that would allow for the calculation of an abundance estimate. This resulted in Chapman estimates of 593 (SD = 551) and 230 (SD = 107), respectively.

Detailed analysis of samples collected in Cumberland Sound near Pangnirtung (2011, 2012; n = 167; 139 individuals) revealed 35 matches, of which 19 matches were on the same day of the same year (oversampling, most times this was recognized in the field notes). There were no recaptures between years, although the number of consecutive years of sampling is low. In 2011 there was a single within-year, between-day recapture and in 2012 there were two within-year, between-day recaptures. This resulted in Chapman estimates of 188 (SD = 163) and 566 (SD = NA), respectively. There were no within-year recaptures in 2008 and therefore no estimate could be calculated.

Percent error in replicated 2008-2012 dataset was negligible in that no errors detected in samples replicated once and a very low rate of dropout (0.024) was observed in positive control samples that were replicated three or more times. In old to new correction (and assuming new is correct) average error rate across 21 loci was 0.064 (range 0.0 to 0.30). Although this suggest a high error rate the use of a minimum of 10 loci out of 21 loci to determine matched sampled would tend to reject matches not create more.

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<sup>1</sup> If an animal was sampled multiple times in the same location and year it was only counted as a single within-year within-location recapture for the summary of all samples. Therefore, the number of recaptures ( $41+13+6=60$ ) does not equal the number of samples minus the number of individuals ( $714-642=72$ ).

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## DISCUSSION

Results indicated that with the increased efforts of recent years to collect bowhead whale biopsy samples, science should be able to provide abundance estimates that could be useful for co-management. In addition to estimation of abundance, mark/recapture methods provide for the opportunity to assess trends if several years of intensive sampling are conducted at regular intervals similar to successful capture-mark-recapture programs in other regions (Boulanger et al. 2004, Coster et al. 2011, Wiig et al. 2011). One of the major benefits of this method of sample collection is that local hunters or community members could lead sampling with minor training and cost and thereby gain the engagement of co-managers. This has been done previously on bowhead whales in Disko Bay, Greenland (Wiig et al. 2011) and killer whales in Cumberland Sound (Cory Matthews pers. comm.).

The majority of between-location recaptures have occurred between northern Foxe Basin (Igloolik) and Cumberland Sound (Pangnirtung). This could be due to more whales moving between the two locations or simply a result of increased effort in these areas. Also of interest is the uni-directional pattern of whales marked in Foxe Basin being recaptured in Cumberland Sound but no whales marked elsewhere (including West Greenland<sup>2</sup>) were recaptured in Igloolik. This is where the sampling effort has been greatest both in number of years and number of samples obtained.

These movements correspond with satellite telemetry data which shows whales tagged in northern Foxe Basin moving into the Gulf of Boothia and Prince Regent Inlet when the ice breaks and farther as the year proceeds (Bernard LeBlanc pers. comm.). The whales tagged in Cumberland Sound seem to have a more complex movement pattern. In July 2006, nine whales were tagged in Cumberland Sound, six provided data for more than one month. Of those six whales, four migrated out of the Sound within the first few weeks of tag deployment. Conversely, in August 2012, 11 whales were tagged in Cumberland Sound and only two of them migrated out of the Sound (Bernard LeBlanc pers. comm.). The expectation would be that resident and non-resident Cumberland Sound bowhead whales would have equal opportunities to be biopsied or tagged and more Cumberland Sound whales would be identified in other areas of the Arctic. Results provided here and preliminary telemetry results suggest that there may be a segment of the EC-WG population that remains year round in Cumberland Sound. A more fine-scale examination of the capture-recapture locations and field notes may help resolve this matter. Although the abundance estimates are similar in magnitude it may be more telling that there were recaptures in two of three years (2008 had only 3 samples) in Cumberland Sound while there were recaptures in two of five years in northern Foxe Basin (2008 had only 6 samples). This may suggest a smaller population or more sedentary population in Cumberland Sound and a larger or more transient population in Foxe Basin.

The local population size estimates should be treated as an exercise only as some of the model assumptions are likely violated, specifically equal capture probabilities for all animals and closure of the sampling area (Boulanger and McLellan 2001, McKelvey and Schwartz 2004). Koski and Ferguson (2012) raise the concern that given the known summer segregation of age, sex, and reproductive class and given the sampling regime, that females would be over represented in the collections. Examination of bowhead samples in the DFO collection reveals that this is not the case. 425 samples have sex data associated with them and there is no bias evident (198F:213M:14U). However, further examination of the field notes may indicate reproductive class biases.

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<sup>2</sup> 78 West Greenland samples were also investigated and there were no matches between WG and EC.

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In 2013 samples were collected and an aerial survey was conducted in Cumberland Sound (not at the same time but both in the summer). This may allow for some comparison of local population estimates. Ultimately, local estimates of bowhead whales may not be informative for population estimates or the management of the stock given the high mobility of this species. Capture-mark-recapture abundance analyses are increasingly sophisticated and these data should be able to be used to generate models and estimates in the program MARK (White and Burnham 1999). Adding samples from 2013 and from west Greenland could lead to a robust abundance estimate for the eastern Canadian-west Greenland bowhead whale population. The simple local estimates reported here are not useful for management and only serve to illustrate that the data is sufficient to continue. They could be compared to recent aerial survey estimates to validate both methods.

In some years and in some locations oversampling has occurred resulting in increased sampling and processing costs with no improvement in the estimate. This issue was also encountered by Wiig et al. (2011) and is likely due to the lack of distinguishing features on many bowhead whales, especially younger animals. The field notes from one year did indicate that many of the same-day resampling was intentional or at least was recognized by the sampling team. Reducing this would lead to a more efficient program and reduce any negative impacts that pursuit and biopsy may have on the whales.

Overall, genetic capture mark recapture could be an effective way to track population abundance and trends in bowhead whales. This method has the potential to be cost effective, engage communities, and provide unbiased population estimates for the co-management process.

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